Remarks

Applicants have amended the claims to expedite prosecution of preferred embodiments. Specifically, claims 1, 9, 12 and 17 have been amended to make explicit that the method is directed to determining haplotypes wherein the polymorphic markers are about one or more kilo base pairs apart from each other. Support for this amendment can be found throughout the specification, and for example in paragraphs [010] and [011]. Claims 1, 9, 12, and 17 have been also amended to make explicit that which was implicit, namely that the term "primer pair" refers to two primers that amplify a nucleic acid region between them, i.e. a region is flanked by the primer pair. Claims 1, 9, 12 and 17 have also been amended to specify at least three polymorphic markers are being looked at. Support for this amendment can be found, for example in paragraph [016]. In addition, claims 1, 9, 12, and 17 have been amended to clarify the claims regarding the different primers, nucleic acid regions and polymorphic markers by designating them as "first" and "second" primers, nucleic acid regions and polymorphic markers. Claims 1, 9, 12 and 17 have been further amended to address issues related to antecedent basis. These amendments are clerical. These amendments are clerical. Claim 17 has been further amended to clarify the method as determining a haplotype of a nucleic acid comprising methylation sites. Support for this amendment can be found, for example in paragraphs [039] and [066]-[067]. Claims 2, 10, 13, and 18 have been amended to refer to use of a statistical analysis. Support for the amendment can be found, for example in paragraph [018]. Claims 4-6 have been amended to correct the issue of antecedent basis by referring to the polymorphic marker of claim 1 instead of the term "polymorphism." Accordingly, entry of the amendments is respectfully requested.

Applicants have added new claims 19- 24. New claims 19 and 20 are supported, for example in paragraph [016]. New claims 21, 22, and 23 are supported, for example, in paragraph [011], and Figure 3. Support for claim 24 can be found, for example in paragraph [077] and Table 2. Accordingly, entry of claims 19-24 is respectfully requested.

Applicants have amended the specification to remove the embedded hyperlinks. Amendments are clerical and therefore do not introduce new matter and their entry is respectfully requested.

The Examiner objected to claim 17 because of a grammatical error. In view of the amendments to claim 17, Applicants respectfully submit that the objection has been obviated.

Applicants now turn to the specific rejections.

The Examiner rejected claims 1-11, 17 and 18 as allegedly not complying with 35 U.S.C. §112, second paragraph, definiteness requirement. Specifically, the Examiner pointed to lack of antecedent basis for the phrases 'the diluted single nucleotide dilution' and 'the nucleic acid template' in claims 1-11, 'the polymorphism' in claims 4-6. The Examiner also noted that dilution into single nucleotide makes the method of claims 1-11 unclear. In addition, the Examiner noted that the phrase 'amplifying the diluted and undiluted nucleic acid sample', as recited in step (c) renders the claims indefinite.

Applicants have amended claims as described, *supra*.

In view of the amendments, Applicants respectfully submit that the claims now comply with the definiteness requirements of 35 U.S.C. §112, second paragraph. Accordingly, the rejection should be withdrawn.

The Examiner rejected claims 1, 2, and 4-6 under 35 U.S.C. 102(b) as allegedly being anticipated by Ruano et al. (PNAS 87:6296-6300, 1990) ("Ruano").

Applicants respectfully disagree and submit that the rejection be withdrawn for the following reasons.

Ruano only used one primer (GR6 and GR5) pair per reaction. Contrary to the Examiner's interpretation, the pair of primers that Ruano used for allele-specific amplification (e.g., GR1 and GR3) are not a primer pair as the phrase "primer pair" is understood in the art. To make explicit what Applicants mean by the use of the phrase "primer pair", Applicants have amended the claims to make explicit that the phrase is used to refer to two primers that amplify a region between them, i.e. that flank a nucleic acid region. As discussed in the specification, the method taught by Ruano is only applicable over short distances that are under one kilo base pairs long (see, par. [004]). This is because Ruano only uses one primer pair (GR6 and GR5) to amplify only one nucleic acid region per reaction. Thus, the one reaction genotype determination reveals the genotype of markers present only in that single amplicon. The amplicon Ruano teaches is **only 700 bp long**. However, there is **nothing in Ruano that teaches the use of multiple primers in the same reaction**.

The method of Ruano also differs from the present invention because Ruano does not directly amplify the single nucleotide molecule dilution. Ruano explained that they used a so called booster amplification method as described in Ruano and Kidd (see, IDS Ref. No. 29)(see, above Materials and Methods in col. 1, page 6297). Ruano clearly used this method because they did not believe they could amplify the single nucleic acid molecule dilution reliably otherwise. Applicants respectfully submit that a skilled artisan would not have thought of using multiplexing from a single nucleic acid template, specifically if no booster PCT was used. It was also well known that a simple amplification using a single nucleic acid template was not reliable.

Applicants teach that **amplification** can be performed **directly from the single nucleotide molecule dilution**. Applicants also teach that one way to enhance this method is to use amplification of very short fragments to overcome the PCR efficiency problems (see, e.g. par. [077] and new claim 24). This is not taught in Ruano. There is no teaching that in the multiplex haplotyping analysis short fragments would provide a significant advantage as described by the applicants (see, e.g. par [077]). The fact that the Applicants have here shown that multiple primer pairs can be reliably used in the same reaction without such additional booster PCR is surprising and certainly a difference between the method as described in Ruano and the present claims (see, e.g., Figure 3, which shows results from a 5-plex amplification reaction).

Moreover, the present claims make it clear that the polymorphic markers that are part of the genotypes that form the haplotypes are far apart, i.e. they span at least a one or more kilo base pair region. The claims have been amended accordingly.

Accordingly, Applicants respectfully submit that the difference in the present method and Ruano is significant. The method of Ruano lacks the teaching of a step of **amplifying multiple different nucleic acid regions in the same reaction**. Certainly, Ruano does not teach amplifying regions that contain polymorphic markers that far apart in one single reaction or amplicons that are about 100 bp long.

In view of the amendments to the claims and the above, Applicants respectfully submit that the rejection under 35 U.S.C. §102(b) over Ruano should be withdrawn.

The Examiner rejected claim 8 under 35 U.S.C. §103(a) as allegedly being unpatentable over Ruano.

Applicants respectfully disagree and submit that the rejection be withdrawn for the following reasons.

As described, *supra*, Ruano does not teach direct amplification of the diluted nucleic acid sample (i.e. amplification without a booster PCR). In addition, Ruano does not teach use of multiple primer pairs in one reaction. Ruano also does not teach determination of a haplotype using markers that are about one or more kbp apart or the benefit of amplifying multiple short fragments in one reaction. As described, *supra*, it would also not have been intuitive to a skilled artisan to alter the method of Ruano to the method of the present invention because a skilled artisan was aware of the problems with amplification of single nucleic acid molecule dilutions with even one primer pair, not to mention two or more primer pairs.

Therefore, Applicants respectfully submit that the elements of the claim are not taught or suggested by Ruano. Accordingly, Applicants respectfully submit that the rejection of claim 8 under 35 U.S.C. §103(a) should be withdrawn.

The Examiner rejected claim 7 under 35 U.S.C. 103(a) as allegedly being unpatentable over Ruano in view of Ross.

Applicants respectfully disagree and submit that the rejection be withdrawn for the following reasons.

As described, *supra*, Ruano does not teach direct amplification of the diluted nucleic acid sample (i.e. amplification without a booster PCR). In addition, Ruano does not teach use of multiple primer pairs in one reaction. Ruano also does not teach determination of a haplotype using markers that are about one or more kbp apart or the benefit of amplifying multiple short fragments in one reaction. As described, *supra*, it would also not have been intuitive to a skilled artisan to alter the method of Ruano to the method of the present invention because a skilled artisan was aware of the problems with amplification of single nucleic acid molecule dilutions with even one primer pair, not to mention two or more primer pairs.

Ross does not overcome these deficiencies. Ross does not describe single molecule dilution or the use of different primer pairs flanking at least 3 specific regions.

Accordingly, Applicants respectfully submit that the rejection of claim 7 under 35 U.S.C. §103(a) should be withdrawn.

The Examiner rejected claims 3 and 9-11 are rejected under 35 U.S.C. §103(a) as being unpatentable over Ruano in view of Drysdale.

Applicants respectfully disagree and submit that the rejection be withdrawn for the following reasons.

As described, *supra*, Ruano does not teach direct amplification of the diluted nucleic acid sample (i.e. amplification without a booster PCR). In addition, Ruano does not teach use of multiple primer pairs flanking at least 3 different regions in one reaction. Ruano also does not teach determination of a haplotype using markers that are about one or more kbp apart or the benefit of amplifying multiple short fragments in one reaction. As described, *supra*, it would also not have been intuitive to a skilled artisan to alter the method of Ruano to the method of the present invention because a skilled artisan was aware of the problems with amplification of single nucleic acid molecule dilutions with even one primer pair, not to mention two or more primer pairs.

Drysdale does not overcome these deficiencies. All Drysdale teaches is general use of haplotypes in the prediction of a phenotypic response to abuterol.

Accordingly, Applicants respectfully submit that the rejection of claims 3 and 9-11 under 35 U.S.C. §103(a) should be withdrawn.

The Examiner rejected claims 12-18 under 35 U.S.C. §103(a) as being allegedly unpatentable over Ruano in view of Rein.

Applicants respectfully disagree and submit that the rejection be withdrawn for the following reasons.

As described, *supra*, Ruano does not teach direct amplification of the diluted nucleic acid sample (i.e. amplification without a booster PCR). In addition, Ruano does not teach use of multiple primer pairs in one reaction. Ruano also does not teach determination of a haplotype using markers that are about one or more kbp apart or the benefit of amplifying multiple short fragments in one reaction. As described, *supra*, it would also not have been intuitive to a skilled artisan to alter the method of Ruano to the method of the present invention because the skilled artisan was aware of the problems with amplification of single nucleic acid molecule dilutions with even one primer pair, not to mention two or more primer pairs.

Rein does not overcome these deficiencies. All Rein teaches are methods for identification of 5-methylcytocine and related modifications in DNA genomes.

Accordingly, Applicants respectfully submit that the rejection of claims 12-18 under 35 U.S.C. §103(a) should be withdrawn.

In view of the foregoing, the Applicants respectfully submit that all claims are in condition for allowance. Early and favorable action is respectfully requested.

In the event that any additional fees are required, the PTO is authorized to charge our deposit account No. 50-0850.

Date: April 7, 2008

Respectfully submitted,

/Leena H. Karttunen/

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